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THE EFFECT OF P-CHLOROMERCURIBENZOATE ON AMOEBOID MOVEMENT, FLAGELLAR MOVEMENT AND GLIDING MOVEMENT

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It has been shown (Abe, 1959, 1963) that the rotational protoplasmic streaming observed in plant cells is dependent upon sulfhydryl (–SH) groups in the protoplasm. In addition, De Robertis and Peluffo (1951) reported an intimate relation between the motility of a flagellated bacterium, *Proteus vulgaris*, and –SH groups. Therefore, it seemed worthwhile to investigate the relationship between –SH groups and other types of protoplasmic motility. Amoeboid movement, ciliary or flagellar movement and gliding movement have been dealt with in this report.

Materials and Methods

Amoeboid movement: A strain of *Amoeba proteus* was chosen as an example of an organism exhibiting amoeboid movement. This strain was found to be abundant in cultures of *Elodea*, and was cultivated by the addition of some uncooked rice grains. The specimens were prepared in the following manner.

The amoebae were sucked up, together with the original culture medium, in a pipette and dropped onto the center of a glass slide. In order to avoid mechanical pressure from the coverslip, a pair of thin glass rods about 10 mm. in length and 0.5 mm. in diameter were laid on the slide. Then, the coverslip was put on the drop carefully. Excessive water was removed with a piece of filter paper.

Flagellar movement: Flagellated cells of coenobia of *Pandorina morum* served as a material exhibiting flagellar movement. The material was collected from a rice paddy in a suburb of Osaka.

As these organisms move freely and actively through an aqueous medium, the preparation used for the amoebae is impracticable for their observation. In this case, only one glass rod was used as a support. A coverslip was brought in position after a drop of suspension of coenobia was carefully placed at the center of the slide. A piece of filter paper was next brought in contact with the edge of the coverslip resting on the glass slide, so that only the suspension medium would be sucked off. In this procedure, coenobia in the suspension were carried along with the stream of water and were caught and held in the wedge-shaped space between

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the coverslip and slide. By this means, we obtained a preparation with a slightly slanted coverslip, under which locomotion was prevented, but with which active movement of the flagella could be observed. Whenever exchange of the medium was required, a solution was made to flow in the same direction in order not to refloat the coverslip and not to release the trapped organisms.

Gliding movement: A species of *Oscillatoria* with a comparatively large diameter (about 16 μ) and a species of pinnated marine diatom, *Nitzschia longissima*, were used as materials. The cells of *Oscillatoria* used in the present study are blue-green and disk-shaped, and the ratio of the diameter to the length is about 7. Each filament tip of this species is straight.

The filaments of *Oscillatoria* are well known to show a bending oscillatory motion, but when a short filament (300 μ– 400 μ in length) is isolated free from others, this motion is transformed into one of gliding along the axis of the filament, so that the speed of motion becomes measurable. The speed and direction of the movement of the filament, however, undergo changes even under constant environmental conditions, and temporary cessation often takes place during the movement. Therefore, in order to compare the behavior of the filament under a particular experimental condition with the control, continuous measurements were made for as long as 2–4 hours.

As the motion of *Nitzschia* is slow and takes a fairly straight path, it is not difficult to determine the changes in velocity of locomotion in this organism with an ocular micrometer and stopwatch.

Reagents used throughout this investigation were commercial p-chloromercuribenzoate (PCMB) and L-cysteine supplied from Wako Pure Chemical Industries, Ltd. Since PCMB is only slightly soluble in acid or neutral solutions, it was at first dissolved in solutions of sodium hydroxide and subsequently neutralized with hydrochloric acid. In the present experiments, the sodium concentration was kept at 10^{-2} *M*, 10^{-3} *M* and 10^{-4} *M* according to the concentrations of PCMB. Consequently, for control solutions, 10^{-2} *M*, 10^{-3} *M* and 10^{-4} *M* NaCl solutions were employed. The sodium content of cysteine solutions was made equal to that of the corresponding control solutions. For the marine diatoms, Van't Hoff artificial sea water was used for a basal solution, and solutions of the above reagents were made to contain a concentration of each ion equivalent to that of the artificial sea water.

All of these experiments were performed at room temperature.

Experimental Results

Part 1

Experiments were first carried out with solutions of PCMB at different concentrations. Before each experiment, the medium surrounding the organism was removed with a piece of filter paper, and either saline (in cases of *Amoeba*, *Pandorina* and *Oscillatoria*) or artificial sea water (in the case of *Nitzschia*) was introduced. After having observed the behavior of the organisms under this condition, the solutions were replaced with PCMB solutions containing a certain amount of NaCl or equivalent amount of salts to the Van't Hoff artificial sea water. Concentrations of the PCMB solutions used were 10^{-3} *M*, 10^{-4} *M* and 10^{-5} *M*. The results obtained are summarized in Table I. Detailed data are given in the following text.

TABLE I

The effects of PCMB on amoeboid movement, flagellar movement and gliding movement at different concentrations

Material	Concentrations of PCMB		
	10^{-3} *M*	10^{-4} *M*	10^{-5} *M*
Amoeba	cytolyzed after 10 sec.	cytolyzed after 8 min.	temporary cessation
Pandorina	stopped within several sec.	stopped after 2 min.	no effect
Oscillatoria	stopped within 3 min.	stopped after 10 min.	no effect
Nitzschia		stopped after 1 min.	stopped in 7 min.

Amoeba

(10^{-3} *M*): Immediately after the application of a control solution—10^{-2} *M* NaCl—movement of *Amoeba* became sluggish and the pseudopodia were drawn in. But in a short time, the pseudopodia were re-formed and within 2–3 minutes the organism regained its normal state completely.

After complete recovery, NaCl solution was replaced with 10^{-3} *M* PCMB containing 10^{-2} *M* sodium. Immediately thereafter the boundary between the cell and the external medium became obscure, and the contents of the organism were extruded in about 10 seconds.

(10^{-4} *M*): When the culture medium was exchanged with 10^{-3} *M* NaCl, movement became at first a little sluggish, but it soon recovered. About 30 seconds after admitting 10^{-4} *M* PCMB, the cell gradually became inactive and all pseudopodia were drawn in. Later the organisms became spherical and after 8 minutes they cytolyzed.

(10^{-5} *M*): With 10^{-5} *M* PCMB the effect was much less pronounced. As a control, a solution—10^{-4} *M* NaCl—was first applied in this case, but there was no observable effect. The saline was then replaced with 10^{-5} *M* PCMB containing 10^{-4} *M* sodium. Within 40 seconds all pseudopodia were retracted and locomotion ceased, but in a short time tiny pseudopodia reappeared. In three minutes the motion recovered completely and the organism survived in this medium without any sign of pathological changes.

Pandorina

(10^{-3} *M*): With 10^{-2} *M* NaCl, the flagellar beating of the coenobium remained normal. When the saline was exchanged with 10^{-3} *M* PCMB containing 10^{-2} *M* sodium, flagellar beating came to a standstill in several seconds. Sometimes flagella, which had been beating, were detached from the cell bodies at their basal ends after several seconds.

(10^{-4} *M*): The effect of PCMB on flagellar beating was still to be seen even at a concentration of 10^{-4} *M*; the beating ceased within two minutes.

(10^{-5} *M*): PCMB solution at a concentration of 10^{-5} *M* had no visible effect on flagellar beating.

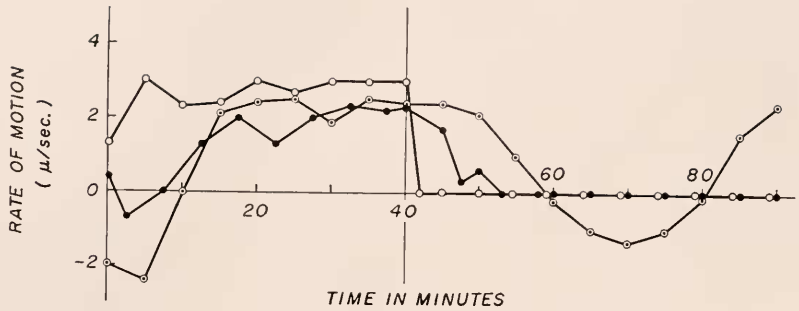

FIGURE 1. The effect of PCMB on the gliding movement of *Oscillatoria* at different concentrations. From 0 to 40 minutes, materials were in NaCl solutions at 10^{-2} *M* (○—○), 10^{-3} *M* (•—•) and 10^{-4} *M* (⊙—⊙); and at 40 minutes, NaCl solutions were replaced with 10^{-3} *M* PCMB + 10^{-2} *M* sodium (○—○), 10^{-4} *M* PCMB + 10^{-3} *M* sodium (•—•) and with 10^{-5} *M* PCMB + 10^{-4} *M* sodium (⊙—⊙).

Oscillatoria

(10^{-3} *M*): As done above, *Oscillatoria* were first placed in NaCl solutions which served as the control. The motion of the filaments, however, was not influenced in NaCl at the concentration of 10^{-2} *M* or less. When 10^{-2} *M* NaCl was replaced with 10^{-3} *M* PCMB containing 10^{-2} *M* sodium, the motion of the filament slowed down and stopped within three minutes and no resumption of motion occurred in the same medium within 20 hours.

(10^{-4} *M*): With 10^{-4} *M* PCMB, the motion decreased, gradually resulting in a complete standstill after 10 minutes. There was no visible change in cell morphology over the observation period of 18 hours.

(10^{-5} *M*): With 10^{-5} *M* PCMB, the gliding motion of the filament was no longer affected at all. These results are illustrated in Figure 1.

Nitzschia

(10^{-4} *M*): The artificial sea water medium was replaced with the artificial sea water containing PCMB. In the plain medium, no change was observable. About

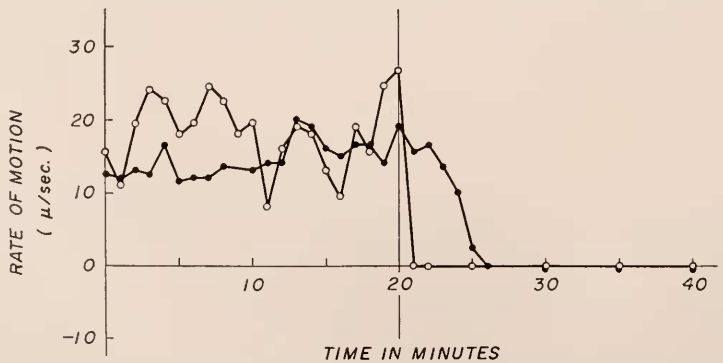

FIGURE 2. The effect of PCMB on the gliding movement of *Nitzschia* at different concentrations. From 0 to 20 minutes, cells were placed in Van't Hoff artificial sea water, and at 20 minutes, medium was replaced with the artificial sea water containing 10^{-4} *M* (○—○) and 10^{-5} *M* (•—•) PCMB.

one minute after the replacement with 10^{-4} *M* PCMB the gliding motion of the organism fell into complete cessation with no spontaneous recovery (Fig. 2).

(10^{-5} *M*): Even with 10^{-5} *M* PCMB, the motion became sluggish and stopped completely in seven minutes (Fig. 2).

Part II

Once the inhibiting concentrations were determined, experiments were carried out to verify the hypothesis that the effect of PCMB was a specific one on sulfhydryl groups. The plan of the experiments with three materials—*Amoeba, Pandorina* and *Oscillatoria*—is illustrated in Table II.

Details of the results of these experiments are given below for each material, the paragraph numbers corresponding to the numbers heading the columns in Table II.

TABLE II

A general outline of the procedure followed and the results obtained in the present experiments. See the text for details

TREATMENT and effect	(1)	(2)	(3)	(4)
	SALINE	SALINE	SALINE	SALINE
	↓	↓	↓	↓
	PCMB	PCMB	PCMB + CYSTEINE	CYSTEINE
	inhibition	inhibition	no inhibition	no visible effect
	↓	↓		
	CYSTEINE	SALINE		
	recovery	no recovery		

Amoeba

(1) About four minutes after treatment with 10^{-4} *M* PCMB, this solution was replaced with 10^{-2} *M* cysteine containing 10^{-3} *M* sodium. Within 60 minutes, no visible change was observed and the cells remained spherical. In such cells, Brownian motion of granules was observed. Then about 70 minutes after replacement, a short pseudopodium was extended, although no conspicuous flow of cytoplasm was observed. After 100 minutes, a pseudopodium with pronounced endoplasmic streaming was formed. By about 160 minutes, complete recovery of amoeboid movement had occurred.

(2) As the control of the former experiment in item (1), PCMB solution was replaced with plain saline instead of a cysteine solution. When 10^{-4} *M* PCMB solution was replaced, about four minutes after its application with 10^{-3} *M* NaCl, 2–3 huge vacuoles appeared in the cells. Thirty minutes later, the cells became spherical, and disintegrated in about 40 minutes.

(3) PCMB and cysteine solutions were mixed before they were applied to the organisms. In this mixed solution, when the amount of the latter exceeds that of the former, the –SH combining power of PCMB was expected to vanish. Control solutions—10^{-3} *M* NaCl—were replaced with a mixed solution containing 5 ×

10^{-5} *M* PCMB, 5×10^{-3} *M* cysteine and 10^{-3} *M* sodium. Under these conditions, the organisms behaved quite normally, and there was no observable effect. In support of this statement it is to be added that the amoebae often phagocystosed ciliated cells in this same medium.

For the sake of contrast, a solution containing 5×10^{-5} *M* PCMB and 10^{-3} *M* sodium was used. About two minutes after application, movement of the organisms almost stopped except for the Brownian motion of the granules inside. Three minutes later, Brownian motion stopped; six minutes later, the organism became spherical and giant vacuoles appeared; 25 minutes later, the organism began to cytolyze.

Pandorina

(1) Immediately after the complete cessation of the flagellar beating with 10^{-3} *M* PCMB, the inhibitor solution was replaced with 10^{-2} *M* cysteine containing 10^{-2} *M* sodium. Then, recovery of the flagellar beating was observed within several minutes. No abnormal behavior was to be seen later in the organisms which had recovered. Even in the coenobium from which flagella had been partially lost by PCMB, flagella left attached recovered their normal beating.

(2) No recovery of flagellar beating took place when 10^{-3} *M* PCMB was replaced with 10^{-2} *M* NaCl solution.

(3) Materials were treated with a mixed solution of 5×10^{-4} *M* PCMB, 5×10^{-3} *M* cysteine and 10^{-2} *M* sodium. With this solution, there was no visible effect on flagellar beating.

On the other hand, when treated with a solution containing 5×10^{-4} *M* PCMB and 10^{-2} *M* sodium, beating stopped within a period of 20–40 seconds and, sometimes, the flagella separated from cell bodies.

(4) Simple application of 10^{-2} *M* cysteine solution with 10^{-2} *M* sodium normal coenobia exerted no effect.

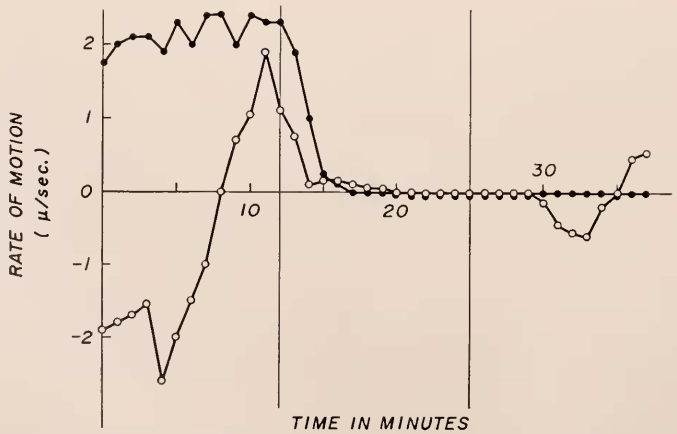

FIGURE 3. Inhibition of the gliding movement of *Oscillatoria* with PCMB and restoration with cysteine. From 0 to 12 minutes, materials were immersed in 10^{-3} *M* NaCl and from 12 to 25 minutes in 10^{-4} *M* PCMB containing 10^{-3} *M* sodium, at 25 minutes, PCMB solution was replaced with 10^{-2} *M* cysteine containing 10^{-3} *M* sodium (○—○) or 10^{-3} *M* NaCl (•—•).

Oscillatoria

(1) About 10 minutes after the complete cessation of gliding motion with 10^{-4} *M* PCMB, the inhibitor solution was replaced with 10^{-2} *M* cysteine containing 10^{-3} *M* sodium. Although in this case a complete recovery was not attained, a partial recovery was observed (Fig. 3).

(2) No sign of recovery was seen in the organism when 10^{-3} *M* NaCl was substituted for 10^{-4} *M* PCMB (Fig. 3).

(3) A mixed solution of 5×10^{-5} *M* PCMB, 5×10^{-3} *M* cysteine and 10^{-3} *M* sodium produced no effect, either on motion or on any other aspect of behavior of the organisms.

In contrast to the above, a solution containing 5×10^{-5} *M* PCMB and 10^{-3} *M* sodium and no cysteine stopped the gliding motion of the filament within fifteen minutes.

(4) In solutions containing 10^{-2} *M* cysteine and 10^{-3} *M* sodium, there was no visible effect on normal organisms.

Discussion

On the basis of the foregoing experiments, it may be concluded that the effect of PCMB on the protoplasmic motility of different types is more or less identical: First, motion becomes sluggish; this is then followed by complete cessation at some particular concentration. Second, motion is restored completely or partially through subsequent application of cysteine in concentrations higher than those of PCMB applied. Third, when the reactivity of PCMB upon −SH groups has been eliminated in advance, through addition of cysteine, PCMB exerts no effect on the cellular motion.

It is well known that PCMB reacts with −SH groups to yield mercaptides. According to Olcott and Fraenkel-Conrat (1947) and Barron (1951), PCMB is the most advantageous of all the known −SH reagents in the following respects: PCMB reacts with −SH groups with high specificity and reacts with no other protein groups; its combination with −SH groups can be thoroughly dissociated with the addition of the reagents having −SH groups in their molecules; these reactions are carried out under physiological conditions. Therefore, it is reasonable to conclude that the cessation and recovery of the movements dealt with in the present paper are closely related to the blocking and liberation of −SH groups in the protoplasm.

In the author's previous work, it was amply verified that the rotational protoplasmic streaming in plant cells has close relation to −SH groups in the protoplasm. In addition, DeRobertis and Peluffo (1951) applied sulfhydryl reagents to the cells of a flagellated bacterium, *Proteus vulgaris*, and reported that the movement of the cells became sluggish and stopped completely under the influence of these reagents, especially PCMB. Recovery of the movement was brought about with subsquent application of cysteine or glutathione, but simple washing with saline or buffer solution did not remove the action of the inhibitors. These results were interpreted by the authors as evidence of the presence of essential −SH groups in the contractile protein of bacterial flagella, or in enzymes especially involved in the mechanism of flagellar motion.

Singer and Barron (1944) showed that PCMB completely inactivated the ATP-ase function of myosin, and subsequent treatment with glutathione restored full activity. Bailey and Perry (1947) have found in addition that the −SH groups of myosin are essential for actomyosin formation. These results point out the significance of −SH groups in muscular contraction. Whether or not the inhibition by PCMB of cellular motions is also due to suppression of ATP-ase activity of a contractile protein is still unknown. But the importance of the −SH groups in the basic mechanism of a variety of protoplasmic motions seems now well established.

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Summary

1. The effect of p-chloromercuribenzoate (PCMB), a specific sulfhydryl reagent, upon several types of cellular motion—amoeboid movement, flagellar movement and gliding movement—was studied.

2. The effect of PCMB on the motions of different types is more or less identical: First, motion becomes sluggish; this is then followed by complete cessation at some particular concentration. Second, motion is restored completely or partially through subsequent application of cysteine in concentrations higher than those of the PCMB previously applied. Third, when the reactivity of PCMB upon −SH groups has been eliminated with cysteine in advance, PCMB exerts no effect on the cellular motion.

3. From the fact that PCMB is bound to −SH groups with high specificity, it is reasonable to conclude that the cessation and recovery of the cellular motion are closely related to the blocking and liberation of −SH groups.

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